Subsequently, microRNA-9 enhances Nr2e1 and Pax6 expressions to promote progenitor proliferation in the ventricular and subventricular zones. MicroRNA-9 targets Nr2e1 mRNA to enhance its protein expression in cooperation with Elavl1 and Msil, and it suppresses the expression of Meis2 that inhibits Pax6 expression. Concomitantly, each cortical layer is reduced and the tangential migration of interneurons into the pallium is impaired in the double mutants. In the subpallium, microRNA-9 suppresses Gsh2 and Foxg1 expression to negatively control progenitor proliferation for the production of the striatum neurons; microRNA-9 also regulates the formation of the pallial/subpallial boundary and ventral pallium through its regulation of Pax6, Nr2e1 and Gsh2 expressions. Furthermore, the globus pallidus is missing, the corridor is malformed and thalamocortical axons and corticofugal axons are miss-routed in the double mutants.

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Program/Abstract # 279

The same enhancer regulates the earliest *Emx2* expression in caudal forebrain primordium, subsequent expression in dorsal telencephalon and later expression in cortical ventricular zone

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We have analyzed *Emx2* enhancers to dissect *Emx2* functions during forebrain development. The FB enhancer we identified immediately 3′ downstream of the last coding exon is well conserved among tetrapods and unexpectedly directed all the *Emx2* expression in the forebrain: caudal forebrain primordium at E8.5, dorsal telencephalon at E9.5–10.5 and cortical ventricular zone later than E12.5. Otx, Tcf and two unknown transcriptional factor binding sites were essential to all these activities. The mutant that lacked this enhancer demonstrated that the *Emx2* expression under the enhancer is essential to diencephalon development and contributes to dorsal telencephalon development and corticogenesis. However, the FB enhancer did not have activities in cortical hem or Cajal–Retzius cells; nor was its activity in the cortex graded. The *Emx2* expression was greatly reduced, but persisted in the telencephalon of the enhancer mutant. There exists another enhancer for the *Emx2* expression unique to mammalian telencephalon.

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Program/Abstract # 280

Characterization of the novel interaction between muskelin and TBX20, a critical cardiogenic transcription factor

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The genetic regulation necessary for cardiogenesis is tightly regulated by transcription factors like TBX20, a member of the T-box (Tbx) transcription factor family. TBX20 is expressed in the heart throughout development and missense mutations in TBX20 have been found in patients with congenital heart defects (CHD). Characterization of modifiers of TBX20 will help elucidate the genetic mechanisms of heart development and CHD. A yeast two-hybrid screen using an embryonic mouse heart cDNA library and TBX20b as bait was used to identify potential modifiers of the TBX20 activity and identified an interaction with muskelin (Mkln), a primarily cytoplasmic protein with potential roles in signal transduction machinery scaffolding and nucleocytoplasmic protein shuttling .The hypothesis of this project is that TBX20 is regulated by muskelin during mouse cardiogenesis. To determine how muskelin regulates the TBX20 activity, the protein interaction, expression patterns, and functional significance of the TBX20b–Mkln interaction will be characterized. In cellular studies, I have shown that muskelin directly binds to the T-box domain of only the TBX20b isoform by its kelch repeats domain. Immunostaining of a myocardium cell line, transfected with tagged TBX20b and muskelin, revealed colocalization in the cytoplasm. Preliminary immunohistochemistry staining on embryonic mouse hearts indicate coexpression in the endocardial valvular and myocardial interventricular cells. Functional significance will be explored using embryonic mouse cardiomyocytes.

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Program/Abstract # 281

Prrxl1 expression in mouse nociceptive neurons is controlled by alternative promoters

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The transcription factor Prrxl1 has a crucial role in the differentiation/survival of nociceptive neurons of dorsal root ganglion (DRG), spinal cord dorsal horn and functionally equivalent supraspinal areas. Nevertheless, our understanding of the transcriptional mechanisms that control the proper spatiotemporal expression of the Prrxl1 gene remains limited. To approach this issue, the 5′-flanking region of Prrxl1 translation start point was analysed by luciferase reporter assays using a DRG-derived neuronal cell line (ND7/23). Three regions displaying promoter activity were identified which are suggestive of alternative promoter usage as a mechanism of control of Prrxl1 expression. Moreover, 5′RACE analysis led us to the identification of Prrxl1 mRNA variants containing distinct 5′UTR regions on Exon 1. These alternative first exons have no consequences in the Prrxl1 open reading frame and therefore are likely involved in differential mRNA stability. In addition, a detailed analysis of the distal promoter revealed the presence of a *bona fide* TATAbox that was validated by EMSA and site-directed mutagenesis. Further analysis of this sequence revealed the presence of two adjacent regulatory elements, one presenting a capability to strongly reduce the combined activity of the three promoters and another one with the potential to inhibit the repressive trait of the former. Altogether, the present results led to the identification of Prrxl1 alternative promoters and some regulatory motifs likely implicated in the modulation of the Prrxl1 expression.

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Program/Abstract # 282

The transcriptional co-repressor TRIM28 is differentially required by KRAB zinc finger proteins during early mammalian embryogenesis

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TRIM28 (aka TIF1β and KAP-1) is a transcriptional co-repressor that functions by inducing heterochromatin formation. The target specificity of TRIM28 is believed to reside in its ability to bind different KRAB domain proteins. Although KRAB zinc finger proteins represent the largest family of transcriptional regulators in mammals, the functions of individual members of this family are largely unknown. Our previous work on *chato*, a mutation in the KRAB zinc finger protein 568, revealed a

requirement for ZFP568 in convergent extension and morphogenesis of embryonic and extraembryonic tissues. Here we present data supporting role for TRIM28 in mediating ZFP568 function; yeast two hybrid assays identified TRIM28 as a partner of ZFP568, while positional cloning of *chatwo*, a hypomorphic allele of TRIM28, provided genetic evidence for the functional significance of a ZFP568/TRIM28 interaction. *chatwo* mutants, isolated in a forward mutagenesis screen, arrest at embryonic day 9 (E9) with defect similar to those of *chato* mutants. The phenotype of *chatwo* embryos contrasts with the early lethality of TRIM28 KO mice that die as pre-gastrula stage embryos (E5.5). Because this data suggests that the *chatwo* hypomorphic allele only affects the functions of TRIM28 required by specific KRAB domain proteins, we investigated whether the *chatwo* mutations disrupt TRIM28 stability, interactions with specific KRAB domain proteins, or recruitment of chromatin modifying enzymes. Results will be presented that support a differential requirement for TRIM28 by distinct KRAB zinc finger proteins during early embryonic development.

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Program/Abstract # 283
A gene regulatory network that underlies the derivation of the anterior neural plate from the epiblast
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The operation of gene regulatory networks that drive developmental processes is reflected in the regulation of core transcription factor genes such as Sox2. The expression of Sox2 in the epiblast and anterior neural plate in the mouse embryo is determined by enhancer N-2. The activation of enhancer N-2 is dependent on a phylogenetically conserved 73-bp core sequence that contains the binding sites for ZIC2, OTX2, and either POU factor OCT3/4 or OCT6. Enhancer N-2 activation requires the binding of ZIC2 and POU factors in both the epiblast and the anterior neural plate, whereas the OTX2 interaction is required only in the latter. This observation, taken together with the expression patterns of these transcription factors, suggests that the transition of major POU factors from OCT3/4 (Class V) to OCT6 (Class III) and the recruitment of OTX2 characterize a shift of the gene regulatory network that underlies the derivation of the anterior neural plate from the epiblast. The impact of POU factor class switching and OTX2 participation on the overall cellular state is currently under investigation by using in vivo and in vitro models.

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Program/Abstract # 284
Mohawk-mediated repression of Sox6 is necessary for the expression of slow myosin heavy chain (Myh7) in differentiated satellite cells
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Mohawk (Mkx) is a member of the TALE superclass of atypical homeobox genes expressed in the embryonic progenitor cell populations of skeletal muscle, tendon and cartilage. We have previously shown that Mkx functions as a potent transcriptional repressor that is capable of inhibiting skeletal muscle differentiation induced by MyoD. To further investigate the role of Mkx during skeletal muscle differentiation, we have isolated muscle satellite cells from Mkx-knockout mice and characterized them in culture under proliferating and differentiated conditions. Microarray analysis revealed that the transcription factor Sox6 is consistently upregulated in proliferating and differentiated Mkx-knockout cells. Sox6 plays a crucial role in muscle fiber type specification through direct repression of slow myosin heavy chain (Myh7). Consistent with this finding, immunostaining of differentiated satellite cells from Mkx-knockout mice revealed that Myh7 expression was reduced, whereas fast myosin heavy chain was unaffected. We have further identified an Mkx-responsive element in the Sox6 locus, suggesting that Mkx may directly repress the transcription of Sox6. Together, this data reveals a novel function for Mkx during the differentiation of skeletal muscle.

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Program/Abstract # 285
Identification of a brain and neural tube specific enhancer associated with the expression of Emx2 during development
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Emx2 is a highly conserved transcription factor expressed in the developing urogenital tract, dorsal limb girdle, and cerebral cortex. Studies of Emx2 null embryos have shown that it is required for normal neuroblast proliferation, migration and differentiation, as well as in the development of the diencephalon. Despite elucidation of some of the developmental functions of the Emx2 gene, few studies exist which characterize regulatory elements controlling the expression of Emx2. We searched the Emx2 locus *in silico*, across divergent species, for conserved noncoding regions (CNR) that might harbor sequences involved in the regulation of Emx2. We identified 34 CNR associated with the Emx2 locus. To determine whether a CNR was functioning as an enhancer element, we transfected chick embryos with a ptk-EGFP reporter construct containing the CNR upstream of a minimal HSV-TK promoter and then screened for an enhancer activity at progressive stages of development by fluorescence microscopy. We found the enhancer activity in a CNR located in the 3′ untranslated region of the Emx2 locus that coincides with the pattern of expression of Emx2 in the developing brain and neural tube. The localized enhancer activity suggests a role for this CNR in the regulation of CNS specific Emx2 expression. Further studies are in progress to confirm this hypothesis and identify specific regulatory proteins that may interact with this region.

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Program/Abstract # 286
Structure of regulatory networks and dynamics of bio-molecules: Predicting unknown from known
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Regulatory relations between biological molecules constitute complex network systems, and realize diverse biological functions through the dynamics of molecular activities. However, we currently